Final Report for CDFA Agreement Number 13-0098-SA
Project Title: Molecular and functional characterization of the putative *Xylella fastidiosa* resistance gene(s) from b43-17 (*V. arizonica*).

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Reporting period: Final report covering period from July 2012 to June 2014

ABSTRACT
Pierce's disease (PD) is a deadly disease of grapevines caused by the bacterial pathogen *Xylella fastidiosa* (Xf). Resistance to PD is present in North American *Vitis* species. Resistance from *V. arizonica* accession b43-17 has been mapped as a single major locus (*PdR1*) onto linkage group 14. The physical mapping of the *PdR1b* allele allowed the identification of potential candidate resistance gene(s). We cloned candidate genes *PdR1b.1,2,4,5* and *6* and generated 5 constructs that were used to transform leaf discs of tobacco and embryogenic callus of *V. vinifera* Chardonnay and Thompson Seedless and *V. rupestris* St. George via *Agrobacterium tumefaciens*. Tobacco and transgenic plants of Chardonnay carrying the candidate genes under the control of 35S CaMV promoter were acclimated for testing against Xf in the greenhouse. Nine to ten independent lines of each gene were pinprick inoculated in two basal nodes with 10ul of 10^8 CFU/ml of Xf Beringer strain. Symptoms based on leaf scorch and cane maturation index (CMI) were scored 3 months after inoculation. Transgenic tobacco plants exhibited promising results at symptom level with candidate genes *PdR1b.1 and 6*. Some transgenic Chardonnay also had phenotype scores comparable to those of resistant biocontrols, however Xf counts evaluated by ELISA showed high concentrations in most transgenic lines. Chardonnay PdR1b5-7a showed the lowest CMI and bacteria concentrations among the transgenics, although not as low as the resistant biocontrols. Transgenic plants of Chardonnay-PdR1b2, Thompson Seedless and St. George will be tested next. Some lines transformed with *PdR1b.6* displayed a dwarf phenotype indicating that the constitutive expression of this gene is affecting normal growth. Meristematic bulks of Thompson Seedless, Chardonnay and St. George have been produced to accelerate genetic transformation via organogenesis.

LAYPERSON SUMMARY
We maintain and characterize many populations while breeding PD resistant wine grapes, some of which have been used to develop genetic maps. These maps were used to identify genetic markers that are tightly linked with PD resistance, and which have allowed classical breeding to be greatly expedited through marker-assisted selection. Genetic maps allow the construction of physical maps to identify resistance genes (Riaz et al. 2008; Riaz et al. 2009). The physical map of the b43-17 resistance region allowed us to identify candidate genes responsible for PD resistance. Comparisons with plant genomes indicated that multiple tandem repeats of the disease resistance gene family Receptor-like proteins with leucine rich repeates (LRR) domains were present in the resistance region. This category of genes is involved in the recognition of microbes and in the initiation of defense responses (Bent and Mackey 2007). We completed the cloning of five candidate genes: *PdR1b.1,2,4,5* and *6* and confirmed their sequence. We also developed embryogenic callus cultures of PD susceptible Chardonnay and Thompson Seedless and rootstock St. George for genetic transformation to verify candidate PD resistance gene function. *PdR1b.1,2,4,5* and *6* were used in transformation of tobacco and grape. Transgenic tobacco plants were tested against Xf in the greenhouse and promising results were obtained with *PdR1b.1* and *6* candidate genes. Transgenic grape plants have been acclimated to greenhouse conditions and Xf inoculations have been initiated. Screening of two sets of plants of Chardonnay, comprising a total of 9-10 lines for each gene, was completed in February and July 2014. Xf counts showed high concentrations in most transgenic lines. PdR1b.5-7a showed the least severe disease symptoms and bacteria concentrations among the transgenics, although not as low as the resistant biocontrols. Testing of transformed Chardonnay-*PdR1b2,* Thompson Seedless and St George is scheduled next. Although the

current transgenic grape plants were produced using the traditional procedure, we are also testing another technique to speed the development of transgenic tissue from meristematic bulks that will allow *PdR1* gene candidates to be tested faster.

This research is focused on demonstrating whether PD resistance genes developed from genetic and physical mapping efforts function when transformed into susceptible host plants. These transformations are underway in tobacco (an easily used model system) and susceptible grape (Chardonnay, Thompson Seedless and St. George). These studies will lay the foundation to understanding how these resistance genes work, and may provide a tool to genetically engineer grape resistance genes into susceptible grapevines.

INTRODUCTION

New cultivars bred to resist *Xylella fastidiosa* infection and subsequent expression of PD symptoms will provide long-term sustainable control of PD. Disease resistant cultivars can be obtained by conventional breeding through the introgression of resistance from North American *Vitis* species into elite *V. vinifera* wine and table grapes. Another approach is "cisgenesis" – the transformation of elite *V. vinifera* varieties with grape resistance genes and their native promoters, cloned from disease resistant American *Vitis* species. The cisgenic approach may have a more limited impact on the genome of the elite *V. vinifera* parent since single genes from the *Vitis* species genome would be added to the elite parent, thus limiting the impact on its fruit and wine quality while making it PD resistant. The cisgene approach in grapes could be considered to be similar to the natural clonal variation that exists in many wine grape cultivars. This linkage-drag-free approach is attractive, and also allows the opportunity to stack additional resistance genes from other *Vitis* sources, even if these genes originate from the same chromosomal position in different species or accessions (Jacobsen and Hutten 2006). The physical mapping of the resistance region from *V. arizonica/candicans* b43-17, *PdR1*, allowed the identification of potential candidate resistance gene(s). Preliminary comparisons indicated that the *PdR1* region contains multiple tandem repeats of Serine Threonine Protein Kinase with a LRR domain (STPK-LRR) gene family. This category of genes belongs to a group involved in plant resistance. Their defense mechanism is based on compounds involved in the recognition of microbe-associated molecular patterns (MAMP) like compounds, which initiate a defense response (Bent and Mackey 2007). In order to gain insight and to verify the function of resistance gene(s), cloning and functional characterization is required. In this report, we present the progress on the cloning and testing of five candidate resistance genes.

OBJECTIVES

1. Cloning, structural analysis and gene annotation via comparison of the *PdR1b* locus to the susceptible Pinot noir genome sequence using the assembled sequence of the BAC clone H64J14.
2. Expression studies of candidate genes. **Previously reported on.**
3. Complementation tests of candidate gene(s) to test their function using: *Agrobacterium*-mediated transformation of the susceptible *Vitis* cultivars (Chardonnay and Thompson Seedless, and the rootstock St. George); and transformation of tobacco.

RESULTS AND DISCUSSION

Objective 1. Cloning, structural analysis and gene annotation via comparison of the *PdR1b* locus to the susceptible Pinot noir genome sequence using the assembled sequence of the BAC clone H64J14

A refined genetic map of chromosome 14, which contains the PD resistance locus, was generated from three grape mapping populations derived from *V. arizonica/candicans* b43-17. The resistance locus segregates as a single dominant gene and mapped as *PdR1a* in the F1 selection 8909-17 and as *PdR1b* in its sibling F8909-08. Clone H69J14 from a b43-17 BAC library, containing both markers flanking the *PdR1b* resistance locus, was sequenced using 454 and paired end Sanger sequencing on two different libraries (fosmid and shotgun). The assembly of the sequence data generated 10 contigs, with a portion of the sequence remaining unassembled. Analysis of assembled and unassembled sequences revealed the presence of four candidate genes, *PdR1b.1 – 4*, which appear to code for receptor-like proteins, a class of resistance proteins. Earlier in 2014, we employed PAC BIO RSII sequencing approach to sequence H69J14 and three other overlapping BAC clones. The assembled sequence data generated 604Kb long fragment with out any gaps (Fig. 1).

In comparison to the susceptible sequence, the resistant line has 126 Kb more sequence than susceptible PN40024 corresponding sequence that is 491.2 Kb. In next step, we will fully annotate the sequence, carry out comparative sequence analysis (manuscript in progress) and proceed to promoter isolation and characterization of the resistant genes. The results of this work will feed into the project "Molecular-functional approach to facilitate the discovery of noval Xylella fastidiosa resistance gene(s) and markers in Native American species" that is continuation of this work and funded by the CDFA PD/GWSS from 2014 - 2017. We have amplified and confirmed the sequences of five candidate genes: *PdR1b.1-2-4-5-6*. *PdR1b.1* (P1) is the largest gene (3198 bp), and shares a high degree of homology with *PdR1b.2* (P2), *4* (P4) *and 5* (P5). *PdR1b.6* (P6) is significantly different from the other four. It has a kinase domain, which suggests it might be involved in PD resistance in combination with P1 or one of the other candidates.

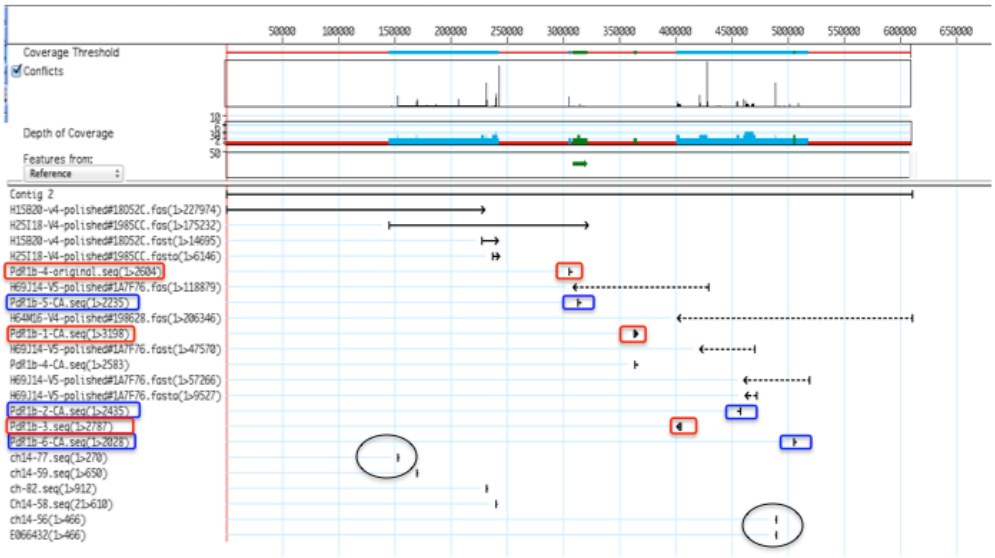

Fig. 1. Assembled sequence of four BAC clones including H69J14 for 604Kb region of the resistant line. Blue and red boxes are the respective identified Open Reading Frames of candidate resistant genes. Circles shows the placement of markers that flank the resistant locus.

Objective 3. Complementation tests of candidate gene(s) to test their function using: *Agrobacterium*-mediated transformation of the susceptible *Vitis* cultivars (Chardonnay and Thompson Seedless, and the rootstock St. George); and transformation of tobacco –
Once the gene constructs are completed, they must be tested to see if they maintain their function and provide resistance. This is done by inserting the genes into a susceptible plant and testing to see if the insertion results in resistant plants. Currently, the most widely used method for the production of transgenic/cisgenic grapes is based on *Agrobacterium* transformation followed by regeneration of plants from embryogenic callus. We have established cultures of pre-embryogenic callus derived from anthers of *V. vinifera* Thompson Seedless (TS) and Chardonnay (CH) and the rootstock *V. rupestris* St. George (SG) that have been used for transformation (Agüero et al. 2006).

PdR1b candidate genes were amplified using Phusion high-fidelity DNA polymerase (Finnzymes), cloned into pGEM-T easy vector (Promega) and sequenced at UC Davis Sequencing Facility. After sequence verification, genes were sub-cloned into binary vector pCambia 1303 (www.cambia.org) containing the 35S cauliflower mosaic virus promoter, the nopaline synthase terminator and an *hptII*-selectable marker gene. P1 was also sub-cloned into binary vector pDU99.2215 containing an *ntpII*-selectable marker gene. The resulting plasmids were transformed into disarmed *A. tumefaciens* EHA105 pCH32 by electroporation and used for transformation of CH, TS, and SG.

Pre-embryogenic calli of TS, CH and SG transformed with the 5 candidate genes were selected in medium with antibiotics, then sub-cultured to germination medium for plant regeneration. The presence

of the genes was checked in callus cultures through PCR and tested again in plants transferred to the greenhouse. For each gene, we attempted to produce, at least 10 independent lines that will be subsequently propagated clonally to 6 plants per line and tested under greenhouse conditions. Table 1 shows the number of independent lines that have been obtained at present. Genomic DNA was isolated from these plants with DNeasy Plant Mini Kit (Qiagen). A primer that binds the CaMV 35S promoter and a primer that binds the coding region of each *PdR1b* candidate were used in combination for PCR amplification to verify the presence of the transgene in the plants transferred to the greenhouse (Fig. 2).

Two sets of screenings have been completed in February 2014 and July 2014. A third screening is underway that is scheduled to end in November 2014. Each line was multiplied through green cuttings to produce 6 replicates. They were cut back to two buds and re-grown. Inoculations via the pinprick procedure were performed 8 weeks after the grapevines had been cut back, when all plants had reached a height of about 1m. Plants were inoculated below and above the node within 5 to 10 cm from the base of the main shoot; using 10 µL of Beringer strain (OD600=0.25) each time. PD resistance was analyzed through phenotype scoring and ELISA. Symptoms of PD were evaluated using a 0-5 score for leaf scorch-leaf loss (LS-LL) and a 0-6 score for cane maturation index (CMI). For ELISA, plants were sampled 12 weeks post-inoculation by taking 0.5 g sections of stem tissue from 30 cm above the point of inoculation (Krivanek and Walker 2005, Krivanek et al. 2005).

The first screening included lines of Chardonnay transformed with P1, P4, P5 and P6; with 5 independent lines per gene. All transgenic lines tested in this experiment displayed disease symptoms with different degrees of intensity. Line CH P5-7a had the lowest bacteria concentrations among the transgenics, although not as low as the resistant biocontrols. It also exhibited low cane maturation index (CMI) and leaf scorching-leaf loss scores (LS-LL) (Table 2). The second round of screenings started in March 2014 and ended in July 2014. It included the remaining lines of CH P1, P4, P5 and P6, plus 5 lines of TS P6 and one line of SG P6. Symptom scoring is shown in Table 3. This was a severe screen, with high CMI and LS-LL scores, not only in transgenics but biocontrols as well. ELISA tests determined that bacteria concentrations were high in all transgenic lines. Both SG P6-20 and SG-untransformed grouped with the resistant genotypes. A third round of screenings started in July to test of CH- P2 and the rest of the TS and SG lines. Transgene expression was confirmed in randomly picked plants through qPCR analysis. Several lines of CH transformed with P6 exhibited an altered phenotype characterized by stunted growth (Fig. 3). Gene expression analysis through qPCR showed that lines with higher expression levels had lower main shoot growth (Fig. 4). Blast analysis of P6 showed high degree of homology with lysine motif receptor-like kinases (LYK), which have been implicated in the recognition of bacterial peptidoglycans (Gust et al. 2012).

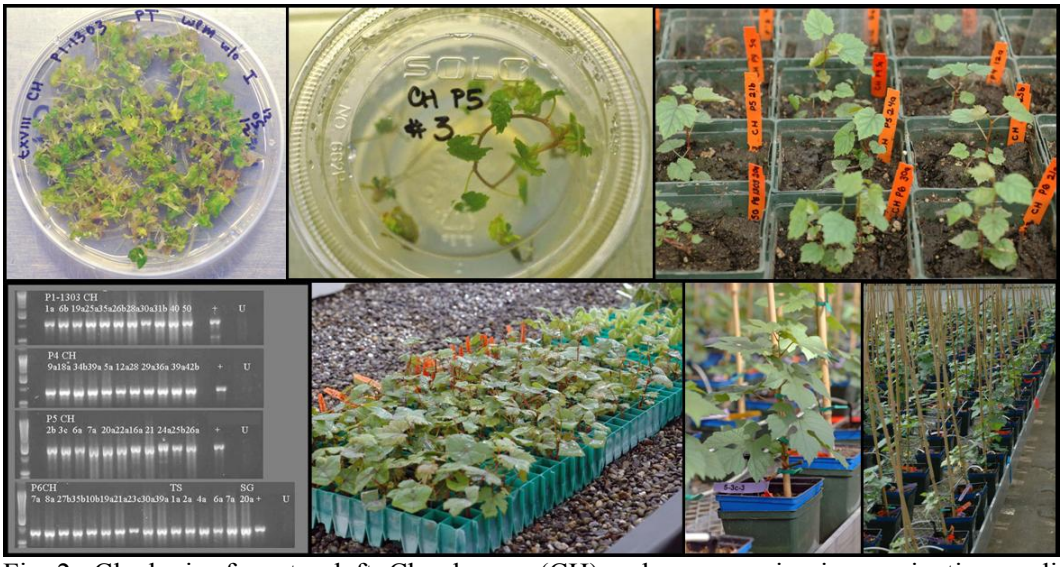

Fig. 2. Clockwise from top left: Chardonnay (CH) embryos growing in germination medium, regenerated plantlets growing *in vitro*, *in vitro* plants transferred to substrate in greenhouse, transgene detection through PCR, green cuttings in mist bed, plants after being cut back prior to inoculation.

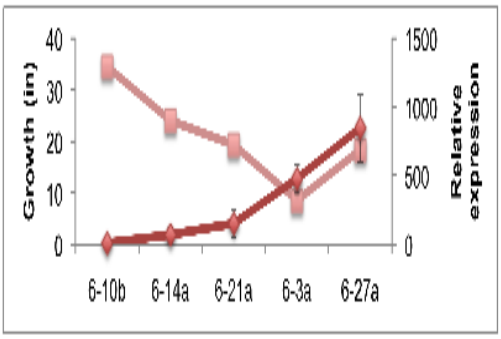

Fig. 3. Dwarf phenotype of P6 transgenic (left) vs. phenotype of untransformed Chardonnay (right).

Fig. 4**.** Expression levels of P6 (red**)** and growth of main shoot (pink) in five independent lines of Chardonnay. Expression levels were analyzed by qPCR, relative to un-transformed Chardonnay.

Table 1. Number of independent lines produced until June 2014; lines in the greenhouse are shown in parentheses. CH = Chardonnay; TS = Thompson Seedless; SG = St. George

	CH	TS	SG
P1 pDU 99.2215	4 (3)	0	0
P1 pCambia1303	15 (10)	5 (2)	13 (10)
P2 pCambia1303	6 (2)	0	0
P4 pCambia1303	20 (10)	7 (2)	4 (4)
P5 pCambia1303	13 (10)	5 (2)	2 (2)
P6 pCambia1303	18 (10)	8 (7)	2 (1)

Table 2. Greenhouse screen results for Chardonnay transformed with P1, P4, P5 and P6. U0505-01 is the resistant biocontrol. U0505-35, U0505-22, b43-17, Roucaneuf, and Blanc du Bois are additional biocontrols. CH uninoculated is the negative control

Genotype	Reps	Geometric mean (cfu/ml)	Mean (ln cfu/ml)	Std Error (ln cfu/ml)	CMI Mean	CMI Std Err	LS-LL Mean	LS-LL Std Err
CH uninoc	6	9,897	9.2	0.0	0.2	0.2	0.9	0.2
b43-17	6	10,232	9.2	0.0	1.8	0.2	2.7	0.4
Roucaneuf	6	16,592	9.7	0.5	0.0	0.0	0.7	0.2
U0505-01	6	27,356	10.2	0.5	0.8	0.7	1.7	0.3
Blanc du Bois	5	43,478	10.7	0.8	2.2	0.4	2.4	0.2
U0505-35	6	107,291	11.6	0.8	0.2	0.2	2.2	0.3
CH P5-7a	6	705,527	13.5	1.0	0.3	0.2	2.2	0.5
CH P1-19a	6	1,518,601	14.2	0.5	1.3	0.6	2.7	0.3
CH P4-9a	5	1,559,694	14.3	0.4	1.0	0.3	2.4	0.2
CH P6-14a	6	1,764,363	14.4	0.4	1.5	0.5	2.2	0.3
CH P1U-20a	6	1,794,075	14.4	0.4	1.1	0.5	2.7	0.3
CH P4-39a	4	2,032,953	14.5	0.4	1.6	0.9	2.8	0.3
CH P5-6a	6	2,303,638	14.7	0.4	2.0	0.4	2.7	0.2
CH P1U-10c	6	2,421,748	14.7	0.5	2.0	0.7	2.7	0.3
CH P5-2b	6	2,421,748	14.7	0.5	1.8	0.6	2.2	0.3
CH P4-34b	5	2,520,581	14.7	0.3	1.5	0.6	2.8	0.4
CH P6-8a	5	2,520,581	14.7	0.4	3.6	0.4	2.8	0.4
CH P5-20a	6	3,007,741	14.9	0.4	2.0	0.7	2.5	0.3

CH P1-6b	5	3,078,645	14.9	0.3	3.4	0.2	2.4	0.2
CH untrans-0	6	3,324,068	15.0	0.4	2.0	0.5	2.5	0.3
CH P1-35a	6	3,379,708	15.0	0.3	2.5	0.6	2.7	0.3
CH untrans-2	6	3,436,623	15.1	0.4	3.8	0.2	2.5	0.2
U0505-22	6	3,494,497	15.1	0.5	2.0	0.7	1.6	0.3
CH P5-22a	6	3,673,664	15.1	0.4	1.1	0.5	2.3	0.4
CH untrans-1	6	3,926,661	15.2	0.3	2.0	0.6	2.5	0.2
CH P6-7a	3	4,127,985	15.2	0.5	2.0	0.6	3.0	0.6
CH P6-35b	3	4,268,188	15.3	0.4	1.5	0.5	1.7	0.3
CH P1U-18b	5	4,325,334	15.3	0.3	2.4	0.5	2.2	0.4
CH P1-1a	5	4,685,579	15.4	0.3	1.7	0.4	2.4	0.2
CH P1-25a	6	5,213,177	15.5	0.1	2.3	0.8	2.7	0.2
CH P4-18a	5	5,722,979	15.6	0.1	1.1	0.5	3.0	0.3
CH P5-3c	4	6,261,936	15.7	0.1	1.5	1.0	3.0	0.4
CH P6-27b	5	6,582,993	15.7	0.0	3.8	0.2	3.0	0.3

Table 3. Greenhouse screen results for Chardonnay transformed with P1, P4, P5 and P6, and Thompson Seedless and St. George transformed with P6. U0505-01 is the resistant biocontrols. U0505-35, U0505-22, b43-17, and Roucaneuf are additional biocontrols. CH uninoculated is the negative control.

Genotype	Reps	Geometric mean (cfu/ml)	Mean (ln cfu/ml)	Std Error (ln cfu/ml)	CMI Mean	CMI Std Err	LS-LL Mean	LS-LL Std Err
CH uninoc	6	10,757	9.3	0.1	0,2	0,2	3,8	0,5
b43-17	6	40,135	10.6	0.4	3,3	0,2	1,5	0,4
SG P6-20	6	393,682	12.9	0.6	4,8	0,2	4,2	0,4
U0505-01	6	638,387	13.4	0.7	1,3	0,3	3,0	0,0
SG untrans	6	984,609	13.8	0.3	3,8	0,5	3,5	0,4
U0505-35	6	2,303,638	14.7	0.4	2,7	0,6	3,2	0,2
Roucaneuf	6	5,300,438	15.5	0.1	4,3	0,3	5,0	0,0
CH P4-12a	6	5,666,034	15.6	0.2	3,8	0,2	4,0	0,4
CH P5-16a	6	5,761,452	15.6	0.1	3,7	0,3	4,2	0,4
U0505-22	6	5,761,452	15.6	0.1	4,2	0,2	3,8	0,3
CH P5-25b	6	5,956,538	15.6	0.1	3,3	0,3	4,0	0,3
CH P1-28a	6	6,158,230	15.6	0.0	3,7	0,2	4,3	0,3
CH P1-40	6	6,261,936	15.7	0.1	3,7	0,2	3,8	0,3
CH P4-36a	6	6,367,389	15.7	0.0	4,2	0,4	4,5	0,2
CH P6-19a	6	6,473,969	15.7	0.0	4,0	0,0	3,8	0,4
CH untrans-1	6	6,582,993	15.7	0.0	3,0	0,4	3,8	0,3
CH P1-26b	5	6,582,993	15.7	0.0	4,0	0,0	3,8	0,2
CH P1-30a	6	6,582,993	15.7	0.0	3,7	0,3	4,0	0,3
CH P4-28a	6	6,582,993	15.7	0.0	3,3	0,3	4,0	0,3
CH P4-42b	6	6,582,993	15.7	0.0	3,5	0,2	4,2	0,2
CH P4-5a	6	6,582,993	15.7	0.0	3,7	0,3	4,0	0,4
CH P5-21b	6	6,582,993	15.7	0.0	3,3	0,2	4,2	0,2
CH P5-24a	6	6,582,993	15.7	0.0	3,3	0,5	4,5	0,2
CH P6-30a	6	6,582,993	15.7	0.0	4,0	0,0	4,2	0,3
CH P6-39a	6	6,582,993	15.7	0.0	3,7	0,3	4,0	0,0
CH untrans-0	6	6,582,993	15.7	0.0	3,8	0,2	4,3	0,2

TS untrans	6	6,582,993	15.7	0.0	5,7	0,2	5,0	0,0
TS P6-1a	6	6,582,993	15.7	0.0	5,0	0,4	5,0	0,0
TS P6-2a	6	6,582,993	15.7	0.0	5,7	0,2	5,0	0,0
TS P6-4a	6	6,582,993	15.7	0.0	5,8	0,2	5,0	0,0
TS P6-6a	6	6,582,993	15.7	0.0	6,0	0,0	5,0	0,0
TS P6-7a	6	6,582,993	15.7	0.0	5,8	0,2	5,0	0,0

Tobacco transformation

To speed the functional analysis, MSc student Carolina Bistue also transformed the tobacco variety SR1, which was recently demonstrated to be a susceptible host for *X. fastidiosa* and is much easier and quicker to transform and test (Francis et al. 2008). Transgenic tobacco plants carrying each candidate gene (9-10 independent lines per gene) were produced at the UC Davis Transformation Facility and multiplied *in vitro* in our lab. No significant differences were observed in stem Xf counts between untransformed controls and transformed plants 12 weeks post inoculation (Fig. 5). However, candidate genes P1 and P6 displayed significantly lower symptoms compared to the untransformed controls (Fig. 6). Expression analysis by real-time PCR confirmed expression of both genes.

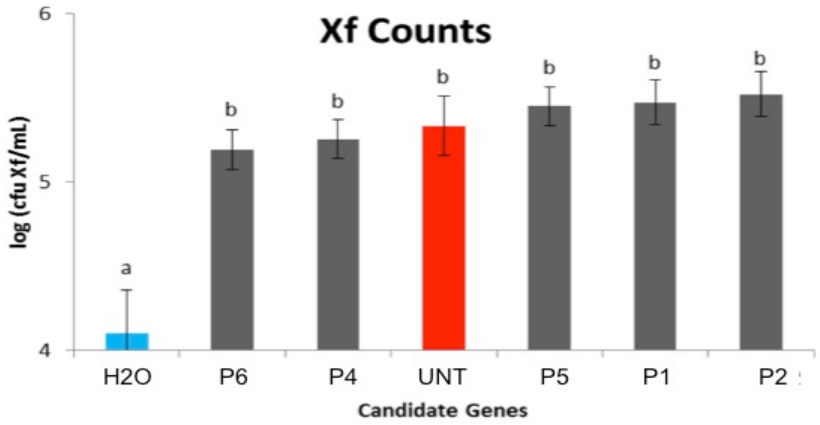

Fig. 5. ELISA results for transformed candidate genes as well as negative controls (H2O) and positive controls (UNT). Samples were stem sections collected aprox. 50 cm above the POI.

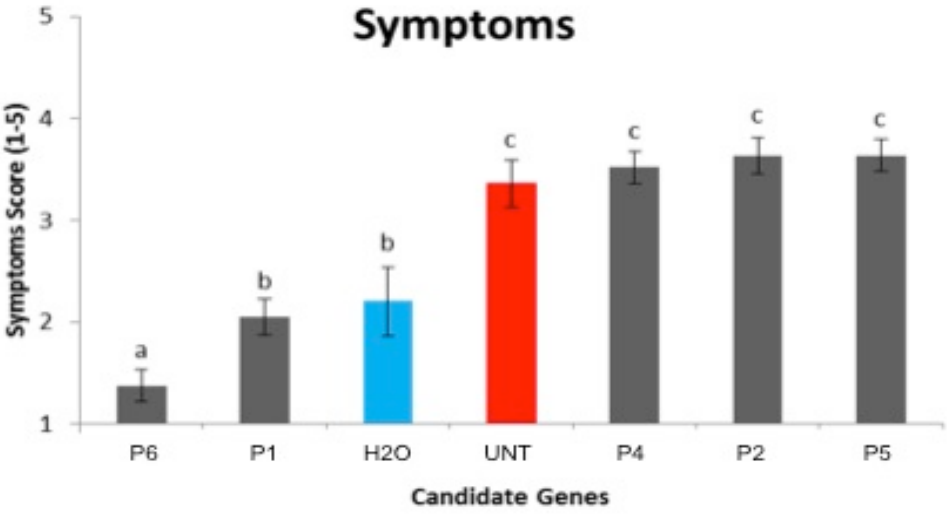

Fig. 6. Symptom results for transformed candidate genes as well as negative controls (H2O) and positive controls (UNT).

Genetic transformation via organogenesis

Inoculation with *A. tumefaciens* of meristematic bulks (MB) is being tested as an alternative transformation technique via organogenesis to reduce the time needed to produce transgenic grapes (Mezzetti et al. 2002). In our lab, transgenic plants of Thompson Seedless expressing GFP were produced in 3 months using MB and kanamycin as the selective agent. Based on these results, Thompson Seedless MB slices were inoculated with *A. tumefaciens* carrying P5 in pCAMBIA 1303 using 3 initial levels of hygromicin: 5, 10 and 15 ug/ml. Since no regeneration was produced at any of the concentrations tested, experiments assaying 0 ug / ml in the first subculture after inoculation, followed by 2.5 ug /ml hygromicin were initiated. Two, out of 50 initial MB, regenerated at this lower concentration, but efforts to establish regenerated plants were unsuccessful.

The partial success obtained with the use of hygromicin and the production of MB of CH and SG, prompted PhD student Xiaoqing Xie to test different hormone ratios to adapt the process to these cultivars and study the use of different antibiotics. She has developed protocols to produce MB of TS, CH, SG and 101-14 Mgt (Fig. 7) and has transformed them with *A. tumefaciens* carrying plasmids pCambia 1303 and pCambia 2303 to compare the use of hygromicin and kanamycin as selectable markers (Fig. 8). Regeneration efficiency has been greatly improved by delaying selection 1 or 2 weeks (Table 4), although this might increase the risk of producing chimeric plants, which will be checked through the *gus* reporter gene.

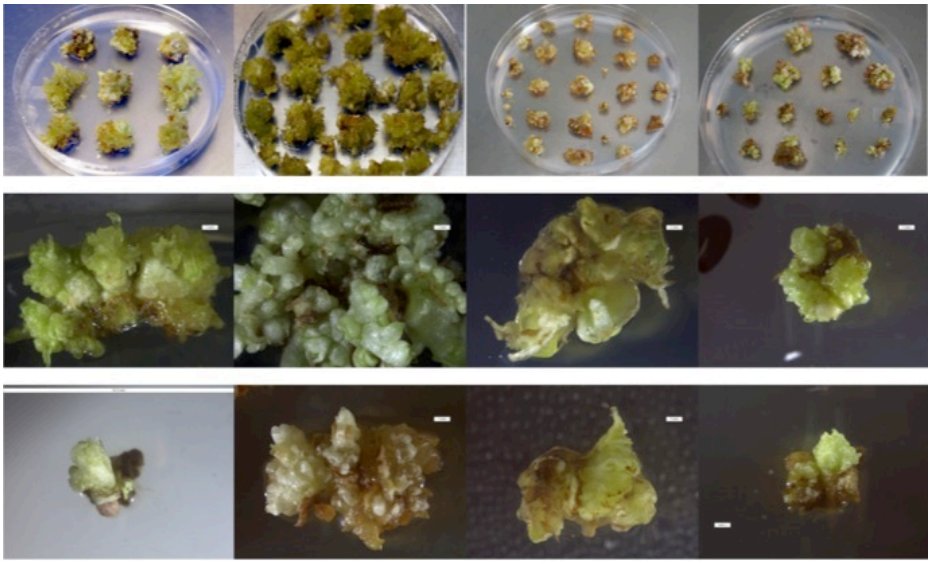

Fig. 7. Genetic transformation of CH, TS, SG and 101-14 via organogenesis: row 1, Meristematic Bulk (MB) induction; row 2, MB before transformation; row 3, shoot regeneration from transgenic meristematic slices; column 1, CH column 2, TS; column 3, SG; column 4, 101-14 Mgt.

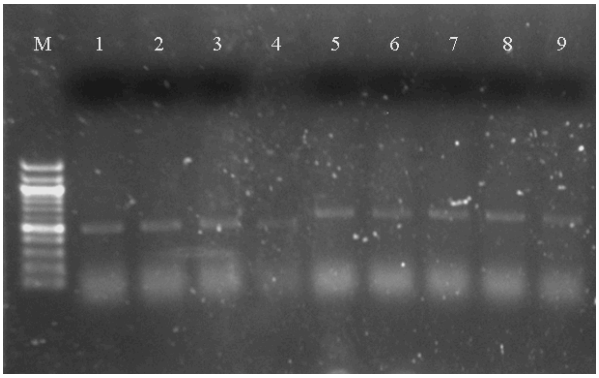

Fig. 8. PCR analysis of tissue from regenerated shoots from Meristematic Bulks inoculated with *A. tumefaciens* carrying pCambia 2301-kan (lanes 1- 4) and pCambia 1303-hygr (lanes 5-9).

Table 4. Percentage survival rate after one month of selection in kanamycin (KAN) or hygromicin (HYG), calculated relative to the number of treated explants. Each value represents the mean ± SE of three different experiments

Weeks before selection	Antibiotics (µg/ml)	Genotype			
		TS	CH	SG	101-14 Mgt
0 W	KAN 100	42.34±2.2	51.67±18.37	41.56±0.56	29.9±6.68
0 W	HYG 2.0	15.15±0.61	16.26±1.25	14.95±0.74	14.44±1.36
1 W	KAN 100	90.67±1.89			
1 W	HYG 2	77.14±4.28			
2 W	KAN 100	97.33±1.15			
2 W	HYG 2	92.8±6.23			

Funding for this project was received from the CDFA PD/GWSS Board

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